PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
D 2145 PCT /1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08570	01/09/2000	10/09/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant EPIDAUROS BIOTECHNOLOGIE AG et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/02/2001	12.12.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bradbrook, D Telephone No. +49 89 2399 7413

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/08570

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-48 as originally filed

Claims, No.:

1-40 as received on 28/11/2001 with letter of 28/11/2001

Drawings, sheets:

1/9-9/9 as originally filed

Sequence listing part of the description, pages:

1-41, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 32,33(in full); 1-31,34-40(in part).

because:

   ☒ the said international application, or the said claims Nos. 27,28 for IA relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 32,33(in full); 1-31,34-40(in part).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-40(in part).

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-31,34-40
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-31,34-40
Industrial applicability (IA)	Yes:	Claims	1-26,29-31,34-40
	No:	Claims	

2. Citations and explanations
   see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Section I Basis of the report

1. Sequence listing pages 1-41 are numbered 49-89.

2. The applicant's observations submitted with the amended claims have been considered in establishing this report.

3. Reference is made to the following documents:

   D1: Westlind et al., Biochem.Biophys.Res.Comm., Vol.259, pp.201-205 (27.05.99);
   D2: WO-A-99 13106 (Axys Pharm.Inc.; 18.03.99).

Section III Non-establishment of opinion

1. As a consequence of the objections expressed in the International Search Report with respect to lack of clarity of claims 1 and 36, examination of specifically identified sequences is restricted thus:
   in claim 1: SEQ ID NOs 54, 55, 129;
   in claim 36: SEQ ID NOs 15, 16, 30, 31, 54, 55, 124, 125, 140, 141.

2. Claims 27 and 28 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion has been formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Section IV Lack of unity of invention

1. The application lacks unity within the meaning of Rule 13.1 PCT.

a. The common concept underlying the application can be defined as the provision of nucleic acid molecules encoding molecular variants of the cytochrome CYP3A4.

b. Document D1 describes the evaluation of testosterone hydroxylase activity of CYP3A4 from 46 different human liver samples, which lead to the identification of

three variant nucleotide exchanges, all causing a mutation of A to G at -290 (CYP3A4-V) in the nifedipine specific element; the importance of this polymorphism was evaluated. D2 teaches a method for detecting a polymorphism in CYP3A4 in an individual (cf p.15, l.29 - p.17, l.24; Table 3). The presence of the predisposing polymorphism is indicative of an alteration in CYP3A4 expression or activity. The method is useful to screen patients for altered metabolism of CYP3A4 substrates, potential drug-drug interactions and adverse side-effects and diseases that result from environmental or occupational exposure to toxins; the variant nucleic acids may be used to establish animal, cellular and in vitro cell-free models for drug metabolism.

c. In the light of D1 or D2, the aforementioned common concept is not novel. Therefore, the problem underlying the application may be redefined as the provision of further isolated nucleic acids encoding variants of CYP3A4.

d. Since the single general concept is not novel, the requirement of Rule 13.1 PCT is not fulfilled and hence there is lack of unity. Neither the description nor the claims revealed any further features that could be considered special in the sense of Rule 13.2 PCT. Therefore, the subject-matters of the different groups of invention are not so linked by a single general inventive concept as required by Art.17(3)(a) and Rule 13.1 PCT.

e. The separate groups of invention are:

Invention 1: A molecular variant M1 of cytochrome CYP3A4, having a nucleotide substitution at position 6004, and a molecular variant of cytochrome CYP3A7, having a nucleotide substitution at position 1229; their corresponding nucleotide and protein sequences; vectors, host cells, antibodies, transgenic non-human animals, pharmaceutical compositions, probes or oligonucleotides thereof/therewith; methods of diagnosis or identification of inhibitors capable of modulating the activity of said molecular variant of CYP3A4 or CYP3A7. (Claims 1-40 in part)

Invention 2-18: A molecular variant of cytochrome CYP3A4, respectively

designated M2, M3, M4, M5, M6, M7, M8, M10, M11, M12, M13, M14, M15, M16, M17, M18 or M19 (cf Table 3); its corresponding nucleotide and protein sequence; vectors, host cells, antibodies, transgenic non-human animals, pharmaceutical compositions, probes or oligonucleotides thereof/therewith; methods of diagnosis or identification of inhibitors capable of modulating the activity of the respective molecular variant of CYP3A4. (Claims 1-40 in part)

2. The subject-matter of invention 1 only was searched and is the subject of examination in this report.

3. It is noted that polymorphic forms of CYP3A4 and CYP3A7 were grouped together as one invention for the international search, and this designation has been retained for the international examination.

Section V Reasoned statement

1. Novelty (Article 33(2) PCT) and inventive step (Article 33(3) PCT)

a. The present invention is based on the discovery of two polymorphisms: in the CYP3A4 gene, a G/A substitution in exon 3 at nucleotide position 6004, giving rise to an amino acid substitution of Gly / Asp in the protein; in the CYP3A7 gene, a C or G in exon 11 at position 1229, giving Thr / Arg in the protein.

c. Neither of these polymorphisms is identified in the prior art. Therefore, the subject-matter of claims 1-31 and 34-40, insofar as searched and examined, appears to be novel.

d. However, the mere discovery of a polymorphism in a known gene is not in itself inventive: polymorphisms are widespread throughout the human genome, and have been identified previously in the CYP3A4 gene (cf D1 and D2). The identification of another polymorphism without the demonstration of an unexpected technical effect of the polymorphism would not appear to solve a technical problem. It is disclosed in the application that the G6004A substitution in

the CYP3A4 gene results in altered activity of the encoded enzyme with respect to two of the three substrates tested (cf p.38-39: bridging paragraph); according to Example 6, the expression of the M1 mutant in a bacterial system is similar to that of the wild-type. The implications for a subject carrying the altered enzyme appear not to have been addressed, and it cannot be assumed that the altered activity would inevitably be a cause of cancer or any other condition. This appears to be the case particularly with CYP3A4, which shows broad substrate specificity and considerable variation in expression and catalytic activity in the general population (cf D1: abstract; present description: p.3, para.2). As such, knowledge of the existence of the altered enzyme in some individuals does not appear to solve a problem, particularly with respect to diagnosis or treatment of disease.

Moreover, there is no indication in the application as to the effect of the T1229R polymorphism on the expression or activity of the CYP3A7 protein, or the phenotypic effect on an individual carrying the variant protein. As no effect has been identified, it appears that no problem is solved.

e. Therefore, claims 1-31 and 34-40 appear not to be inventive.

2. Industrial applicability (Article 33(4) PCT)

For the assessment of the present claims 27 and 28 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Section VI Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO-A-00 24926	04.05.00	22.10.99	23.10.98*

*priority not checked

The examination report has been based on an assumed valid priority for the present application. Should the priority of the present application not be valid, the above document would be relevant with respect to novelty and inventive step (Article 33(2) and (3) PCT). Furthermore, should the present application enter the national or regional phase, the above document could be relevant to the question of novelty.

Section VII

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D2 is not mentioned in the description, nor are these documents identified therein.

Section VIII

1. The following objections are under Article 6 PCT:

a. The preferred embodiment defined by claim 3 relates to a result to be achieved, viz the expected effect of a polymorphism in a gene; in this case the subject-matter should be defined by the polymorphisms which cause the required effect, as it is not clear from the application that the polymorphisms in question result in altered expression of the genes concerned (cf e.g. Example 6).

b. Claims which rely on a causative association between the polymorphisms and a disease are speculative as there is no support in the application for such. Moreover, such claims are not properly disclosed, contrary to the requirements of Article 5 PCT. As has been discussed in Section V, the application provides no guidance to the skilled person as to a possible relationship between the polymorphisms of the invention and the occurrence of a condition in individuals carrying the different polymorphic forms. The altered activity with respect to two substrates (of three tested) appears to be insufficient in this respect, particularly in view of the broad substrate specificity of the CYP3A4 protein. As such, there is considered to be an undue burden on the skilled person to determine how the polymorphisms relate to a particular medical condition.

PCT Patent application R[redacted]P00/08570
EPIDAUROS BIOTECHN[redacted]OGIE AG et al.
Our Ref.: D2145 PCT/1

Claims

1. A polynucleotide selected from the group consisting of:
   (a) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 54, 55, 58, 59, 62, 63, 66, 67, 70, 71, 74, 75, 78, 79, 82, 83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103, 106, 107, 110, 111, 118, 119, 122, 123, 126, 127, 128, 134, 138, 144, 146, 148, 150, 151, 152, 153, 154, 156, 157, 159, 161, 162, 163, 164 or 171;
   (b) a polynucleotide encoding a polypeptide having the amino acid sequence of any one of SEQ ID NO: 129, 135, 139, 145, 147, 155, 158, 160 or 172;
   (c) a polynucleotide encoding a CYP3A4 or CYP3A7 polypeptide, wherein said polynucleotide is having at a position corresponding to any one of position 6004, 13908, 14292, 14304, 14323, 14329, 14357, 15753, 20230, 21867, 21868, 21896, 22026, 22041, 23081, 23172, 25925 or 25958 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as position 1) or at a position corresponding to position 1229 of the CYP3A7 (Accession No: gi4503232) a nucleotide exchange, a nucleotide deletion, an additional nucleotide or an additional nucleotide and a nucleotide exchange, wherein said nucleotide deletion at a position corresponding to position 23172 is not resulting in an M to T amino acid substitutuion or is not a T to C nucleotide exchange;
   (d) a polynucleotide encoding an CYP3A4 or CYP3A7 polypeptide, wherein said polynucleotide is having at a position corresponding to any one of position 6004, 13908, 14292, 20230 or 21868 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as position 1) an A, at a position corresponding to any one of position 14323, 14329, 21867, 21896, 22026, 22041, 23081 or 25925 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as

position 1) a T, at a position corresponding to any one of position 14357, 15753 or 25958 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as position 1) a G, at a position corresponding to any one of position 14304 of the CYP3A4 gene (Accession No: AF280107, whereby the nucleotide A of the first ATG encoding the CYP3A4 protein has been taken as position 1) a C or at a position corresponding to position 1229 of the CYP3A7 gene (Accession No: gi4503232) a G;

(e) a polynucleotide encoding an CYP3A4 polypeptide, wherein said polypeptide comprises an amino acid substitution at any one of position 56, 130, 170, 174, 363, 373, 416 or 445 of the CYP3A4 polypeptide (Accession No: AF280107), wherein said substitution at a position corresponding to position 445 is not M to T; and

(f) a polynucleotide encoding an CYP3A4 or CYP3A7 polypeptide, wherein said polypeptide comprises an amino acid substitution of G to D at position 56, R to Q at position 130, V to I at position 170, D to H at position 174, T to M at position 363, L to F at position 373 or P to L at position 416 of the CYP3A4 polypeptide (Accession No: AF280107) or T to R at position 409 of the CYP3A7 polypeptide (Accession No: gi4503232).

2. The polynucleotide of claim 1, wherein said polynucleotide encodes a variant CYP3A4 or CYP3A7 protein or fragment thereof.

3. The polynucleotide of claim 1 or 2, wherein the nucleotide deletion, addition and/or substitution result in altered expression of the variant CYP3A4 or CYP3A7 gene compared to the corresponding wild type gene.

4. A vector comprising the polynucleotide of any one of claims 1 to 3.

5. The vector of claim 4, wherein the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells.

6. A host cell genetically engineered with the polynucleotide of any one of claims 1 to 3 or the vector of claim 4 or 5.

7. A method for producing a molecular variant CYP3A4 or CYP3A7 protein or fragment thereof comprising
   (a) culturing the host cell of claim 6; and
   (b) recovering said protein or fragment from the culture.

8. A method for producing cells capable of expressing a molecular variant CYP3A4 or CYP3A7 gene comprising genetically engineering cells with the polynucleotide of any one of claims 1 to 3 or the vector of claim 4 or 5.

9. A CYP3A4 or CYP3A7 protein or fragment thereof encoded by the polynucleotide of any one of claims 1 to 3 or obtainable by the method of claim 7 or from cells produced by the method of claim 8.

10. An antibody which binds specifically to the protein of claim 9.

11. The antibody of claim 10 which specifically recognizes an epitope containing one or more amino acid substitution(s) as defined in any one of claims 1 to 3.

12. A nucleic acid molecule complementary to a polynucleotide of any one of claims 1 to 3.

13. A vector comprising the nucleic acid molecule of claim 12.

14. A transgenic non-human animal comprising at least one polynucleotide of any one of claims 1 to 3 or the vector of claim 4 or 5.

15. The transgenic non-human animal of claim 14 further comprising at least one inactivated wild type allele of the CYP3A4 or CYP3A7 gene.

16. The transgenic non-human animal of claim 14 or 15, which is a mouse or a rat.

17. A method of identifying and obtaining a CYP3A4 or CYP3A7 inhibitor capable of modulating the activity of a molecular variant of the CYP3A4 or CYP3A7 gene or its gene product comprising the steps of
    (a) contacting the protein of claim 9 or a cell expressing a molecular variant CYP3A4 or CYP3A7 gene comprising a polynucleotide of any one of claims 1 to 3 in the presence of components capable of providing a detectable signal in response to drug metabolization, with a compound to be screened under conditions to permit CYP3A4- or CYP3A7-mediated drug metabolization, and
    (b) detecting the presence or absence of a signal or increase of a signal generated from the drug metabolization, wherein the presence or increase of the signal is indicative for a putative inhibitor.

18. The method of claim 17 wherein said cell is a cell of claim 6, obtained by the method of claim 8 or is comprised in the transgenic non-human animal of any one of claims 14 to 16.

19. A method of identifying and obtaining an CYP3A4 or CYP3A7 inhibitor capable of modulating the activity of a molecular variant of the CYP3A4 or CYP3A7 gene or its gene product comprising the steps of
    (a) contacting the protein of claim 9 with a first molecule known to be bound by CYP3A4 or CYP3A7 protein to form a first complex of said protein and said first molecule;
    (b) contacting said first complex with a compound to be screened; and
    (c) measuring whether said compound displaces said first molecule from said first complex.

20. The method of claim 19, wherein said measuring step comprises measuring the formation of a second complex of said protein and said compound.

21. The method of claim 19 or 20, wherein said measuring step comprises measuring the amount of said first molecule that is not bound to said protein.

22. The method of any one of claim 19 to 21 wherein said first molecule is nifedipine, rifampicine or corticosterone.

23. The method of any one of claims 19 to 22 wherein said first molecule is labeled.

24. A method of diagnosing a disorder related to the presence of a molecular variant of the CYP3A4 or CYP3A7 gene or susceptibility to such a disorder comprising
    (a) determining the presence of a polynucleotide of any one of claim 1 to 3 in a sample from a subject; and/or
    (b) determining the presence of a protein of claim 9.

25. The method of claim 24, wherein said disorder is cancer.

26. The method of claim 24 or 25 comprising PCR, ligase chain reaction, restriction digestion, direct sequencing, nucleic acid amplification techniques, hybridization techniques or immunoassays.

27. The method of any one of claims 24 to 26, further comprising administering to a subject a medicament to abolish or alleviate said disorder.

28. The method of any one of claims 24 to 27, further comprising introducing
    (i) a functional and expressible wild type CYP3A4 or CYP3A7 gene or

(ii) a nucleotide acid molecule of claim 12 or the vector of claim 14 into cells.

29. A method for the production of a pharmaceutical composition comprising the steps of the method of any one of claims 17 to 23; and
(c) synthesizing and/or formulating the compound identified and obtained in step (b) or a derivative thereof in a pharmaceutically acceptable form.

30. The method claim 29, wherein said compound or derivative thereof is a drug or prodrug in a form suitable for therapeutic application and preventing or ameliorating the disorder of the subject diagnosed in the method of claim 24 or 25.

31. The method of claim 29 or 30 wherein said compound drug or prodrug is a derivative of a medicament as defined in claim 28.

32. An inhibitor identified or obtainable by the method of any one of claims 17 to 23.

33. The inhibitor of claim 32 which binds specifically to the protein of claim 9.

34. Use of an oligo- or polynucleotide for the detection of a polynucleotide of any one of claims 1 to 3 and/or for genotyping of individual CYP3A4 or CYP3A7 alleles.

35. The use of claim 34 wherein said polynucleotide is a polynucleotide of any one of claims 1 to 3 or a nucleic acid molecule of claim 12.

36. The use of claim 34 wherein said oligonucleotide is about 15 to 50 nucleotides in length and comprises the nucleotide sequence of any one of SEQ ID NOS: 1 to 127, 140 or 141 or a complementary sequence.

37. A primer or probe consisting of an oligonucleotide as defined in claim 36.

38. Use of an antibody or a substance capable of binding specifically to the gene product of an CYP3A4 or CYP3A7 gene for the detection of the protein of claim 9, the expression of a molecular variant CYP3A4 or CYP3A7 gene comprising a polynucleotide of any one of claims 1 to 3 and/or for distinguishing CYP3A4 alleles comprising a polynucleotide of any one of claims 1 to 3.

39. A composition comprising the polynucleotide of any one of claims 1 to 3, the vector of claim 4 or 5, the host cell of claim 6 or obtained by the method of claim 8, the protein of claim 9, the antibody of claim 10 or 11, the nucleic acid molecule of claim 12, the vector of claim 13, the inhibitor of claim 32 or the primer or probe of claim 37.

40. The composition of claim 39 which is a diagnostic or a pharmaceutical composition.